Themed Issue: Drug Addiction - From Basic Research to Therapies
Guest Editors - Rao Rapaka and Wolfgang Sadée

Transport Systems for Opioid Peptides in Mammalian Tissues

Submitted: August 9, 2005; Accepted: September 15, 2005; Published: December 29, 2005

Vadivel Ganapathy[1] and Seiji Miyauchi[2]

[1]Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA 30912
[2]Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

Abstract

Transmembrane transport of endogenous as well as synthetic opioid peptides is a critical determinant of pharmacokinetics and biologic efficacy of these peptides. This transport process influences the distribution of opioid peptides across the blood-brain barrier and their elimination from the body. A multitude of transport systems that recognize opioid peptides as substrates have been characterized at the functional level, and these transport systems are expressed differentially at different sites in the body. Many of these transport systems have been identified at the molecular level. These include the H^+-coupled peptide transporters PEPT1 and PEPT2, the adenosine triphosphate-dependent efflux transporters P-glycoprotein and multidrug resistance-related protein 2, and several members of the organic anion-transporting polypeptide gene family. There are however many additional transport systems that are known to transport opioid peptides but their molecular identities still remain unknown.

Keywords: opioid peptides, transmembrane transport, peptide transporters, P-glycoprotein, multidrug resistance-related protein 2, organic anion-transporting polypeptides, sodium-coupled transporters

Introduction

Opioid peptides play a critical role in a variety of biological processes, including analgesia, constipation, respiration, euphoria, sedation, and meiosis.[1] Specific opiate receptors mediate the biological effects of these peptides. There are 4 classes of endogenous opioid peptides: enkephalins, endomorphins, dynorphins, and endorphins. The structures of these peptides are given in Table 1. These peptides are generated in vivo from different precursor proteins (Table 1). These precursors are found primarily in the brain and gastrointestinal tract. Thus, the brain and the gastrointestinal tract represent the primary targets for these opioid peptides.

Corresponding Author: Vadivel Ganapathy, Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA 30912. Tel: (706) 721-7652; Fax: (706) 721-9947; E-mail: vganapat@mail.mcg.edu

In addition to the endogenous opioid peptides, a variety of synthetic opioid peptides and peptide derivatives have been developed for therapeutic or investigational purposes. The biologic efficacy of opioid peptides, either generated endogenously or administered exogenously, depends on various factors, such as the half-life of these peptides in circulation and entry of these peptides to their target sites. One of the major determinants of these processes is the transfer of these peptides across the plasma membrane of cells. Transmembrane transport of opioid peptides has the potential to regulate the access of these peptides to their target sites, to affect the half-life of these peptides by influencing their elimination via tissues such as the liver and kidney, to determine their oral bioavailability via intestinal absorption, and to modulate the concentrations of these peptides in the synapse via reuptake from the synaptic cleft. With regard to the brain, the transfer of these peptides across the blood-brain barrier is critical for their biologic efficacy, and this transfer process involves transmembrane transport in endothelial cells associated with this barrier. Endogenous opioid peptides as well as synthetic opioid peptides and their derivatives exhibit significant hydrophilicity, and therefore the transfer of these peptides across the hydrophobic core of the plasma membrane of mammalian cells cannot occur entirely via diffusion. Specific transport systems have been described in a variety of tissues and cell types for the passage of opioid peptides across the plasma membrane. The purpose of this review is to provide a succinct summary of the most recent findings with regard to the molecular identity and functional characteristics of the transport systems that are known to facilitate the transmembrane transfer of opioid peptides in mammalian cells.

Transport Systems for Opioid Peptides at the Blood-brain Barrier

Transfer of solutes across the blood-brain barrier can occur either in the direction of blood-to-brain or in the direction of brain-to-blood. Methods are available to study these 2 processes independently.[2] There is evidence for the transfer of opioid peptides in either direction. The brain microvascular endothelial cell forms the most prominent component of the blood-brain barrier, and this cell layer is polarized with differential expression of a variety of transport systems

Table 1. Endogenous Opioid Peptides and Their Precursors

Opioid Peptide	Structure	Precursor
Met-enkephalin	YGGFM	Proenkephalin
Leu-enkephalin	YGGFL	Proenkephalin
Octapeptide	YGGFMRGL	Proenkephalin
Heptapeptide	YGGFMRF	Proenkephalin
β-Endorphin	A peptide with 31 amino acids	Pro-opiomelanocortin
Dynorphin 1-8	YGGFLRRI	Prodynorphin
Dynorphin 1-17	YGGFLRRIRPKLKWDNQ	Prodynorphin
α-Neoendorphin	YGGFLRKYPK	Prodynorphin
β-Neoendorphin	YGGFLRKYP	Prodynorphin
Endomorphin 1	YPWF-NH_2	Not known
Endomorphin 2	YPFF-NH_2	Not known

in the luminal surface versus the abluminal surface. These transport systems work in tandem to determine the directionality of the transfer. Even though the evidence for the transfer of opioid peptides via saturable systems in the brain-to-blood direction as well as in the blood-to-brain direction is strong, very little is known on the molecular identity of the transporters responsible for these processes. Four different peptide transport systems have been described for the transfer of peptides across the blood-brain barrier,[3] and these are referred to as PTS-1, PTS-2, PTS-3, and PTS-4. Among these 4, only PTS-1 recognizes opioid peptides as substrates. The substrates of PTS-1 include Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2), Met-enkephalin, Leu-enkephalin, β-casomorphin (Tyr-Pro-Phe-Pro-Gly-Pro-Ile), and dynorphin 1-8. Other opioid peptides such as kyotorphin (Tyr-Arg), β-endorphin, dynorphin A, and dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) do not serve as substrates for PTS-1. Leucine, a bulky hydrophobic amino acid, is an allosteric regulator of this system. This does not however mean that PTS-1 is related to the neutral amino acid transport system L at the molecular level. PTS-1 is most likely located in the capillary endothelial cells of the brain. The transport system appears to mediate facilitated diffusion of its substrates as there is no evidence for the participation of metabolic energy or transmembrane ion gradients in the transport process. A facilitated and energy-independent transport system can potentially function in a bidirectional manner. As the kinetic parameters of enkephalin transfer in the blood-to-brain direction are similar to those for transfer in the brain-to-blood direction, PTS-1 may represent a bidirectional transport system. The molecular nature of this transport system has not yet been established.

Another transport system, distinct from PTS-1, is involved in the brain-to-blood transfer of endomorphin-1 and endomorphin-2.[4] The synthetic opioid peptides DAMGO (Tyr-D-Ala-Gly-*N*-methyl-Phe-glycinol) and DPDPE (Tyr-D-Penicillamine-Gly-Phe-D-Penicillamine) do not interact with this transport system. Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH_2) is structurally related to Tyr-MIF-1, but it interacts with PTS-1 as well as the endomorphin transport system. A recent study has shown that this transport system is located in the basolateral side of cultured brain microvascular endothelial cells.[5] A distinct transport system specific for deltorphin I (Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2) and deltorphin II (Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2) has also been described in brain microvessels.[6] This transport system shows partial dependence on Na^+ but is not concentrative. Furthermore, it does not interact with enkephalins, DAMGO, or DPDPE. Of interest, naloxone but not naltrindole, competes with deltorphins for transport via this system. Preloading of the microvessels with glutamine stimulates the activity of this transport system, but the exact mechanism involved in the process is not known. Since this deltorphin-specific transport system was identified by uptake measurements using intact microvessels,[6] it is likely that the transporter responsible for the process is located in the basolateral side of the endothelial cells.

A Novel Na^+-dependent Active Transport System for Opioid Peptides

Recently we made a serendipitous discovery in a human retinal pigment epithelial cell line (ARPE-19) that there is a Na^+-coupled active transport system specific for opioid peptides.[7] Such a transport system has not been described previously in the literature. The transport system accepts a variety of opioid peptides containing 4 to 13 amino acids. The substrates for this transport system include enkephalins, dynorphins, endorphins, and casomorphins. Various enkephalin derivatives are also recognized by the transport system as is the synthetic opioid peptide deltorphin II. The Michaelis constant for different peptides varies in the range of 0.4 to 40 μmol/L. Dynorphin 1-13 shows highest affinity

for the system. This newly identified opioid transport system is specific for peptides and does not interact with nonpeptide opioids (antagonists) such as naloxone and naltrexone. Of interest, the expression of this transport system in ARPE-19 cells is enhanced markedly by Tat, a protein encoded by the human immunodeficiency virus-1 genome. The functional features of the transport system in control cells and in Tat-expressing cells remain similar, including the Na^+-dependent nature and substrate specificity. Cl^- also plays a role in the transport function, but it is not clear whether the role of this anion in the transport process is direct or indirect. Na^+-activation kinetics of the transport process suggests involvement of at least 3 Na^+ ions per transport cycle. Theoretically, such a transport system is expected to be highly active with an exceptionally marked ability to mediate the cellular uptake of its substrates against a concentration gradient. The unique functional features of this transport system reveal that it is not identical to any of the transport systems that have been identified previously either at the functional level or at the molecular level. The molecular identity of this novel opioid peptide transport system remains unknown.

An exactly identical transport system is also present in SK-N-SH cells, a human neuronal cell line (S. Miyauchi and V. Ganapathy, unpublished data, March, 2004). The characteristics of the system in these cells, monitored with deltorphin II as a substrate, are very similar to those in control and Tat-expressing ARPE-19 cells. The uptake of deltorphin II in SK-N-SH cells is Na^+-dependent and inhibitable by Leu-enkephalin, Met-enkephalin, and dynorphins. The affinities for various endogenous opioid peptides are in the following order: dynorphin 1-13 (0.15 ± 0.01 μM) > dynorphin 1-6 (3.5 ± 0.4 μmol/L) > dynorphin 1-7 (4.6 ± 1.1 μmol/L) > Leu-enkephalin (7.8 ± 1.2 μmol/L) > Met-enkephalin (10.7 ± 3.1 μmol/L) > deltorphin II (19.5 ± 7.8 μmol/L). Of interest, L-lysine is a potent inhibitor of this transport system. Among the naturally occurring amino acids, L-lysine shows the greatest inhibition of deltorphin II uptake. Under similar conditions, L-leucine, L-valine, D-alanine, D-tyrosine, and L-arginine also show significant inhibition, but the potency is much smaller than that seen with L-lysine. D-lysine has no effect, indicating stereoselectivity for the inhibition. Dose-response studies have shown that the inhibition with L-lysine occurs with a K_i value of 160 ± 19 μmol/L. However, L-lysine is not a transportable substrate for the opioid peptide transport system. The inhibition of deltorphin II uptake caused by L-lysine is noncompetitive. L-lysine decreases the maximal velocity of the transport system without affecting the affinity for deltorphin II. Thus, it appears that L-lysine is a blocker of the transport system and that the binding site for L-lysine does not overlap with the substrate binding site on the transporter.

Transport of Opioid Peptides via Cloned Transporters

Peptide transporters PEPT1 and PEPT2

PEPT1 and PEPT2 are H^+-coupled transporters for small peptides consisting of 2 or 3 amino acids.[8] Longer peptides such as tetrapeptides or pentapeptides are not recognized by these transporters. PEPT1 is expressed primarily in the intestine, whereas PEPT2 is expressed in a variety of nonintestinal tissues including the brain. If PEPT1 could transport opioid peptides, it might have relevance to the oral delivery of synthetic opioid peptides for therapeutic purposes. This rationale prompted studies on the handling of opioid peptides by PEPT1.[9] With the human intestinal cell line Caco-2, which expresses a functional PEPT1, as the experimental system, these studies have shown that the synthetic opioid peptides DALDA (dimethyl-Tyr-D-Arg-Phe-Lys-NH_2) is not a substrate for PEPT1. However, the opioid peptide kyotorphin is transported by PEPT2.[10,11] This is not surprising because kyotorphin is a dipeptide; dipeptides are excellent substrates for PEPT2. PEPT1 differs from PEPT2 primarily in substrate affinity, the former being a relatively low-affinity transporter and the latter a high-affinity transporter. Substrate specificity is quite similar for the 2 transporters. Therefore, it is quite possible that kyotorphin is also recognized as a substrate by PEPT1 but with a lower affinity compared with PEPT2. It is unlikely, however, that longer opioid peptides, either endogenous or synthetic, would be recognized as substrates by either of these 2 transporters.

P-glycoprotein

P-glycoprotein is a transporter encoded by the multidrug resistance gene *MDR1*. It is an adenosine triphosphate (ATP)-dependent transporter, and the energy released from the hydrolysis of ATP is coupled to the active efflux of substrates from the cells. The substrate specificity of P-glycoprotein is very broad, including a wide variety of peptides. This efflux transporter is expressed on the luminal side of the endothelial cells, which constitute the blood-brain barrier.[12] Therefore, it is plausible that the transporter might function in the transfer of opioid peptides across the blood-brain barrier in the brain-to-blood direction. Nonpeptide opiates such as morphine[13-15] and asimadoline[16] are effluxed from the brain across the blood-brain barrier via P-glycoprotein. Knockout mice lacking either one or both isoforms of P-glycoprotein ($mdr1a^{-/-}$ mice or mdr1a/b double knockout mice; rodents have 2 genes for P-glycoprotein, whereas humans have only 1 gene for this protein) accumulate more opiates in the brain compared with control mice following intravenous injection and exhibit enhanced sensitivity to opiate-induced analgesia.[13-16] The brain-to-blood transport of endogenous (β-endorphin) as well as synthetic (DPDPE and DAMGO) opioid peptides is impaired when

P-glycoprotein is inhibited, downregulated, or knocked out,[15,17,18] suggesting that these opioid peptides serve as substrates for the efflux transporter. Studies using either transport measurements or stimulation of P-glycoprotein-associated ATPase activity have provided more direct evidence for the transport of DAMGO via P-glycoprotein.[19,20] There is some controversy regarding the role of P-glycoprotein in the handling of endomorphins across the blood-brain barrier. Using DAMGO as a transportable substrate for P-glycoprotein, it has been shown that endomorphin 1 and endomorphin 2 are able to compete with DAMGO for the transport process,[20] but direct measurements of brain-to-blood efflux of endomorphins have provided no evidence for the involvement of P-glycoprotein in the efflux process.[21] These findings may be reconciled if endomorphins function as inhibitors of P-glycoprotein and not as transportable substrates. Enkephalins do not interact with P-glycoprotein.[20,21] It appears that interaction with P-glycoprotein is dictated by specific structural features associated with opioid peptides. Amidation of the C-terminus and presence of at least 2 aromatic amino acids as a part of the peptide structure seem to be essential for recognition by P-glycoprotein.[20]

Organic Anion Transporting Polypeptides

Organic anion transporting polypeptides (human, OATPs; rodents, Oatps) belong to a large gene family consisting of at least 36 members.[22] These transporters mediate the transmembrane transport of anionic substrates of diverse chemical structures and the transport mechanism involves electroneutral anion exchange. Hence, OATPs/Oatps function as bidirectional transporters. The expression of OATPs/Oatps is widespread, detectable in the brain, liver, intestine, kidney, placenta, and eye. Three synthetic opioid peptides, DPDPE, DADLE, and deltorphin II, have been shown to be transported by certain members of the OATP/Oatp family. This includes the rodent Oatp1, Oatp2, Oatp3, and Oatp4[23,24] and human OATP-A, OATP-C, and OATP-8.[24-27] Enkephalins may also be recognized by some of these transporters.[26] The primary location of most of these transporters is the basolateral membrane of hepatocytes.[23,25,28-30] Therefore, these transporters play a critical role in the uptake of opioid peptides by the liver for subsequent elimination into bile. OATP-A is located in brain capillary endothelial cells in humans[24,27] and hence is expected to be an important determinant of transfer of opioid peptides across the blood-brain barrier. There is evidence for marked changes in the ability of OATP-A to transport opioid peptides due to genetic polymorphisms.[27]

Multidrug Resistance-related Protein 2

Elimination of drugs by the liver consists of uptake into hepatocytes across the basolateral membrane followed by secretion of drugs into bile across the canalicular membrane. Opioid peptides enter hepatocytes via OATPs/Oatps. Secretion of these peptides into bile is also likely to be mediated by specific transporters. Even though P-glycoprotein, which is known to transport certain opioid peptides, is located in the canalicular membrane, this transporter does not seem to play a major role in the hepatic elimination of the opioid peptide DPDPE in vivo.[18] Instead, the multidrug resistance-related protein 2 (Mrp2) appears to be important for this process.[31,32]

Conclusions

Multiple transport systems participate in the transfer of endogenous as well as synthetic opioid peptides across the plasma membrane of mammalian cells. This transfer process is an important determinant of pharmacokinetics of these peptides because it influences their distribution across the blood-brain barrier and their hepatic elimination. Even though many of the transport systems that recognize opioid peptides as substrates have been identified at the molecular level, there are some novel opioid peptide transporters that have been characterized only at the functional level. The molecular identity of these transporters still remains unknown.

References

1. Vaccarino AL, Kastin AJ. Endogenous opiates: 2000. *Peptides*. 2001;22:2257-2328.

2. Smith QR. A review of blood-brain barrier transport techniques. *Methods Mol Med*. 2003;89:193-208.

3. Banks WA, Kastin AJ. Peptide transport systems for opiates across the blood-brain barrier. *Am J Physiol*. 1990;259:E1-E10.

4. Kastin AJ, Fasold MB, Smith RR, Horner KA, Zadina JE. Saturable brain-to-blood transport of endomorphins. *Exp Brain Res*. 2001;139:70-75.

5. Somogyvari-Vigh A, Kastin AJ, Liao J, Zadina JE, Pan W. Endomorphins exit the brain by a saturable efflux system at the basolateral surface of cerebral endothelial cells. *Exp Brain Res*. 2004;156:224-230.

6. Fiori A, Cardelli P, Negri L, Savi MR, Strom R, Erspamer V. Deltorphin transport across the blood-brain barrier. *Proc Natl Acad Sci USA*. 1997;94:9469-9474.

7. Hu H, Miyauchi S, Bridges CC, Smith SB, Ganapathy V. Identification of a novel Na^+- and Cl^--coupled transport system for endogenous opioid peptides in retinal pigment epithelium and induction of the transport system by HIV-1 Tat. *Biochem J*. 2003;375:17-22.

8. Leibach FH, Ganapathy V. Peptide transporters in the intestine and the kidney. *Annu Rev Nutr*. 1996;16:99-119.

9. Zhao K, Luo G, Zhao GM, Schiller PW, Szeto HH. Transcellular transport of a highly polar 3+ net charge opioid tetrapeptide. *J Pharmacol Exp Ther*. 2003;304:425-432.

10. Fujita T, Kishida T, Okada N, Ganapathy V, Leibach FH, Yamamoto A. Interaction of kyotorphin and brain peptide transporter in synaptosomes prepared from rat cerebellum: implication of high affinity type H+/peptide transporter PEPT2-mediated transport system. *Neurosci Lett*. 1999;271:117-120.

11. Fujita T, Kishida T, Wada M, et al. Functional characterization of brain peptide transporter in rat cerebral cortex: identification of the high-affinity type H^+/peptide transporter PEPT2. *Brain Res*. 2004;997:52-61.

12. Cordon-Cardo C, O'Brien JP, Casals D, et al. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci USA*. 1989;86:695-698.

13. Schinkel AH, Wagenaar E, van Deemter L, Mol CA, Borst P. Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J Clin Invest*. 1995;96:1698-1705.

14. Thompson SJ, Koszdin K, Bernards CM. Opiate-induced analgesia is increased and prolonged in mice lacking P-glycoprotein. *Anesthesiology*. 2000;92:1392-1399.

15. King M, Su W, Chang A, Zuckerman A, Pasternak GW. Transport of opioids from the brain to the periphery by P-glycoprotein: peripheral actions of central drugs. *Nat Neurosci*. 2001;4:268-274.

16. Jonker JW, Wagenaar E, van Deemter L, et al. Role of blood-brain barrier P-glycoprotein in limiting brain accumulation and sedative side-effects of asimadoline, a peripherally acting analgaesic drug. *Br J Pharmacol*. 1999;127:43-50.

17. Chen C, Pollack GM. Altered disposition and antinociception of [D-penicillamine(2,5)] enkephalin in mdr1a-gene-deficient mice. *J Pharmacol Exp Ther*. 1998;287:545-552.

18. Chen C, Pollack GM. Enhanced antinociception of the model opioid peptide [D-penicillamine] enkephalin by P-glycoprotein modulation. *Pharm Res*. 1999;16:296-301.

19. Sarkadi B, Muller M, Homolya L, et al. Interaction of bioactive hydrophobic peptides with the human multidrug transporter. *FASEB J*. 1994;8:766-770.

20. Oude Elferink RPJ, Zadina J. MDR1 P-glycoprotein transports endogenous opioid peptides. *Peptides*. 2001;22:2015-2020.

21. Kastin AJ, Fasold MB, Zadina JE. Endomorphins, Met-enkephalin, Tyr-MIF-1, and the P-glycoprotein efflux system. *Drug Metab Dispos*. 2002;30:231-234.

22. Hagenbuch B, Meier PJ. Organic anion transporting polypeptides of the OATP/ SLC21 family: phylogenetic classification as OATP/ SLCO superfamily, new nomenclature and molecular/functional properties. *Pflugers Arch*. 2004;447:653-665.

23. Cattori V, van Montfoort JE, Stieger B, et al. Localization of organic anion transporting polypeptide 4 (Oatp4) in rat liver and comparison of its substrate specificity with Oatp1, Oatp2 and Oatp3. *Pflugers Arch*. 2001;443:188-195.

24. Gao B, Hagenbuch B, Kullak-Ublick GA, Benke D, Aguzzi A, Meier PJ. Organic anion-transporting polypeptides mediate transport of opioid peptides across blood-brain barrier. *J Pharmacol Exp Ther*. 2000;294:73-79.

25. Kullak-Ublick GA, Ismair MG, Stieger B, et al. Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with 3 other OATPs of human liver. *Gastroenterology*. 2001;120:525-533.

26. Nozawa T, Tamai I, Sai Y, Nezu J, Tsuji A. Contribution of organic anion transporting polypeptide OATP-C to hepatic elimination of the opioid pentapeptide analogue [D-Ala2, D-Leu5]-enkephalin. *J Pharm Pharmacol*. 2003;55:1013-1020.

27. Lee W, Glaeser H, Smith LH, et al. Polymorphisms in human organic anion-transporting polypeptide 1A2 (OATP1A2): implications for altered drug disposition and central nervous system drug entry. *J Biol Chem*. 2005;280:9610-9617.

28. Reichel C, Gao B, Van Montfoort J, et al. Localization and function of the organic anion-transporting polypeptide Oatp2 in rat liver. *Gastroenterology*. 1999;117:688-695.

29. Konig J, Cui Y, Nies AT, Keppler D. Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. *J Biol Chem*. 2000;275:23161-23168.

30. Konig J, Cui Y, Nies AT, Keppler D. A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. *Am J Physiol Gastrointest Liver Physiol*. 2000;278:G156-G164.

31. Hoffmaster KA, Zamek-Gliszczynski MJ, Pollack GM, Brouwer KL. Hepatobiliary disposition of the metabolically stable opioid peptide [D-Pen2, D-Pen5]-enkephalin (DPDPE): pharmacokinetic consequences of the interplay between multiple transport systems. *J Pharmacol Exp Ther*. 2004;311:1203-1210.

32. Hoffmaster KA, Zamek-Gliszczynski MJ, Pollack GM, Brouwer KL. Multiple transport systems mediate the hepatic uptake and biliary excretion of the metabolically stable opioid peptide [D-penicillamine2,5] enkephalin. *Drug Metab Dispos*. 2005;33:287-293.